Bioactive properties and LC-MS analysis of water dropwort plant

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Abstract
Oenanthe javanica commonly known as water celery or dropwort is a medicinal plant with a wide spectrum of biological properties. Present study was done to evaluate the antibacterial activity of Oenanthe javanica essential oil (OJEO) against Escherichia coli (ATCC 25922) and Bacillus cereus (ATCC 11778). It showed 13±0.57 mm and 12±0.57 mm zone of inhibition against B. cereus and E. coli. Scanning Electron Microscopy imaging was done to confirm the altered bacterial surface morphology on OJEO treatment. OJEO showed 65.09% radical scavenging activity as evaluated by DPPH assay. OJEO also exhibited cytotoxicity against HaCat cell lines by NRU assay.

Liquid chromatography- mass spectrometry (LC-MS) analysis revealed the presence of luteolin, xanthosine, acacetin, scoulerin and other biologically active compounds. In recent decades, antimicrobial resistance has become a worldwide threat. Plant based natural products and their basal formulations can be used as therapeutics, in food preservation and in pharma-based industries.

Keywords: *Oenanthe javanica*, Essential Oil, Antibacterial, Antioxidants, LC-MS.

Introduction

Plants have been seraphic gifts to human civilization since ancient times. Plants provide food, nutrition, shelter, medicine and many more. In traditional medicine, medicinal plants are a central element in treating human diseases. A major population still relies on plant based traditional prophylactics in the modern, advanced medicinal system. In the view site of ethnopharmacological studies, medicinal plants are used as the main objective in the development of drugs to treat human disease ailments. Ethnobotany studies involve the interaction of plants and humans for the use of traditional medicinal plants by indigenous people.

Ethnobotany and ethnopharmacology define traditionally medicinal plants as having potential bioactivity and are being used as beneficial elements and therapeutics against various human and animal diseases[12]. In ancient times, the therapeutic potential of traditional medicinal plants was used as crude drugs made by simple unconventional processes using plants, minerals and animal products, but plants were given priority. Even the word "Drug" is taken from the French word "Drogue" which stands for dry herb and strongly indicates that they were drawn from plant sources. In Ayurveda, Chinese, Unani and Greek medicine, as well as in modern medicine systems, plants play a vital role in pharmacology and pharmacy[27].

Active, effective constituents from plants always contribute to pharmacotherapy and it is one of the main reasons to explore the plant. Natural products have a wide diversity of chemicals that have different drug properties and act as a resource for drug discovery and drug development. Interdisciplinary approaches like ethnobotanical, pharmacological, phytochemical and biotechnological aspects were previously used for successful achievements. Plants are venerable sources of bioactive molecules with various pharmacological attributes including antioxidant, antimicrobial, anti-inflammatory, antiosteoporotic, antimalarial, anti-ageing, antidiabetic, immunomodulator, antihypertension, anticancer, hepatoprotective and others.

Traditional medicine from plants i.e. plants parts or whole plants, is used as a drug or their isolated active components, such as reserpine, atropine, morphine, paclitaxel (taxol), curcumin, demethoxycurcumin, tetrahydrocannabinol (THC), cannabidiol (CBD), digoxin phlorizin etc. These are the milestones in the history of pharmacology and pharmacy[22,24]. Plants have always remained an important source of drugs for all systems of medicine and currently many drugs are derived from both unchanged and small structural modifications to develop novel analogues (modified natural products)[22].

The decades of isolation, identification and identification of prompt therapeutic potential of biologically active molecules from plants have paved way to a new era of drugs. Research efforts have focused on exploring molecules from plants as a potential supply for novel biologically active compounds that could lead to new novel therapeutics[32]. Recently, food borne bacterial pathogens have become a major public health issue responsible for morbidity and mortality in the human population[1]. For decades, researchers have emphasized the search for plants that have an arsenal of eco-friendly antibacterial constituents. Plants' secondary metabolites, such as phenolics, flavonoids, alkaloids, terpenoids received more attention for their bioactivity potential. Secondary metabolites produced by the plants have diverse bioactivity and therapeutic potential such as antimicrobial, anticancer, anti-inflammatory, anti-diabetic, analgesic, sedative, antimalarial and antioxidant[12].

The perennial plant *Oenanthe javanica* (Blume) DC. comes from the Apiaceae family and grows in tropical and temperate parts of Asia. It has been used in the treatment of hepatitis jaundice, diabetes, reducing blood pressure, anti-anaphylaxis, pathogenic wind elimination and hepatoprotective against the hepatitis virus B virus[11]. In present study, *Oenanthe javanica* essential oil was investigated for its bioactive property against *Escherichia coli* (ATCC 25922) and *Bacillus cereus* (ATCC 11778), antiproliferative against HaCat cell line, antioxidant potential and chemical component characterization using LC-MS analysis.

Material and Methods

Plant Sample Collection and Identification: Plant samples were collected from Nichlaul forest, Maharajganj, Uttar Pradesh, India. Plant specimen was further identified by Botanical Survey of India (BSI) Central Regional Centre (CRC), Allahabad, Prayagraj, Uttar Pradesh India.

Extraction of Essential oil: Approximately 200 gram of fresh aerial parts of the plant were cut into tiny pieces and subjected to hydro distillation in a Clevenger type apparatus for 3-5 hours. The essential oils that were extracted were dried over anhydrous Sodium Sulphate (Na_2SO_4) and stored in glass containers at a temperature of 4±1^{0}C for future use.

Bacterial strain culture procurement and storage: Bacterial cell cultures of *Escherichia coli* (ATCC 25922) and *Bacillus cereus* (ATCC 11778) were procured from Hi Media, India.

Antibacterial Screening: Antibacterial activity testing of essential oils was done by Disc Diffusion Assay[5]. 100µl of a fresh overnight bacterial inoculum was disseminated on Muller Hinton Agar (MHA) after it had been adjusted to 0.5 McFarland (108CFU/mL). On MHA plates, a 6 mm sterile Whatmann filter paper disc was impregnated with 5–10 µl of essential oils. Plates were further incubated for 24 hours at 35 °C +/- 2 °C. Finally, the inhibition zone diameter in millimetres was measured.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) determination: MIC was determined according to CLSI guideline by 96 well plates microbroth dilution method[28]. INT (Iodonitrotetrazolium chloride, Hi Media) was used as indicator to check the bacterial growth. To determine the MBC; 10 µl of aliquot placed on NAM plates until no further growth was seen[8].

Scanning Electron Microscopy (SEM): Bacterial cultures were standardized at 0.5 McFarland and subjected to treatment at the minimum inhibitory concentration of OJEO. Centrifugation was performed at 7000 rpm for 30 minutes following treatment. Following centrifugation, the supernatants were discarded and the pellets were washed twice with phosphate buffer (PB). Bacterial pellets were ultimately fixed in Karnovsky's fixative solution and preserved at 4 ± 10°C. Bacterial samples were pre-fixed, suspended in 0.1M phosphate buffer, washed three times and subjected to centrifugation. 1% solution of osmium tetroxide was utilized for post-fixation for one hour. Sample dehydration was performed using varying concentrations of alcohol for a duration of 15 minutes. Samples were coated with gold and Scanning electron microscopy was conducted using the Zeiss Evo-18 microscope.

Neutral Red Uptake (NRU) Assay: Cytotoxicity of OJEO was determined by NRU assay on HaCat cell lines. In 96 well plates, 5000-8000 cells in each wells were cultured for 24 h in DMEM medium supplemented with 10% FBS and 1% antibiotic solution at 37°C with 5% CO_2. Fresh culture medium was introduced to each well of the plate after the medium was removed. The treated plates were incubated for 24 hours after 5 µl of OJEO dilutions were added to the wells. The wells were incubated for 1 hour in the Heal Force-Smartcell CO2 Incubator-Hf-90 after 100 µl of NRU (40 µg/mL in PBS) was added. Lastly, the medium was removed and 100 µl of NRU destain solution was used to dissolve the NRU. Ultimately, the Elisa Plate Reader (iMark BioRad-USA) was employed to read the plates at 550/660 nm[20].

Antioxidant Assay: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was determined with slight modifications according to Brand-Williams et al[7]. 0.1 mM DPPH solution was mixed with various concentrations of 100-500 µg/mL of essential oils and placed in dark for 30 minutes at room temperature. Absorbance was measured at 575 nm. All the experiments were carried out in triplicate.

Liquid chromatography–mass spectrometry (LC-MS):LC-MS analysis was performed on Waters Alliance e2695/HPLC-TQD Mass spectrometer. Spectra were recorded in negative and positive ionization mode between m/z 150 and 2000. Compounds were identified on the basis of online data bank: http://spectra.psc.riken.jp/menta.cgi/respect/index.

Statistical analysis: Data analysis was done on Microsoft Excel and IC_{50} was calculated by using software Graph Pad Prism -6. One way ANOVA was performed on SPSS.

Results and Discussion

Oenanthe javanica showed antibacterial activity and inhibited the growth of food borne bacteria. Disc diffusion assay clearly confirmed the investigations. The MIC value indicated that OJEO was moderately active against the *E. coli* and *Bacillus cereus* having MIC value in between 512 - 2048 µg/ mL according to the cut off points proposed by Tamokou et al[25]. Essential oils from many species of family Apiaceae are recognized to have antibacterial and other medicinal properties. *Coriandrum sativum, Cuminum cyminum* and *Anethum graveolens* essential oils exhibited antibacterial activity against *Bacillus cereus, Staphylococcus aureus* and *Escherichia coli.*

Foeniculum vulgare and *Apium graveolens* EO were active against the *B. cereus* and *E. coli*[19]. *Prangos trifida* was found to inhibit the growth of *S. aureus* and *B. cereus*.

It was also effective against the biofilms formation by *Listeria monocytogenes*, *S. aureus* and *E. coli*[26]. *Oenanthe javanica* had antibacterial activity against *Salmonella* Typhimurium, it exhibited 13.66 mm inhibition zone diameter, 1000 µg/mL and 1500 µg/mL of MIC and MBC respectively[15]. Badalamenti et al[3] investigated that *Prangos ferulacea* essential oil showed antibacterial activity against *Bacillus subtilis*, *B. cereus, S. aureus*, *Salmonella typhimurium, E. coli* DH5α and *Pseudomonas aeruginosa* at MIC value of 100-200 µg/mL.

B. cereus bacterial spores observed by SEM divulged that the control (2% DMSO) had oblong shaped and smooth surface morphology. OJEO treated *B. cereus* spores had reduced morphological surface with shrinkage, damaged and collapsed spores. SEM micrographs illustrated that the *E. coli* control cells (2% DMSO) had smooth surface, rod shaped and uninterrupted cell morphology. OJEO exposed cells had damages surface but not much affected morphology. Figures 1.1, 1.2, 2.1 and 2.2 are the SEM images of *B.cereus* spore and *E. coli* cells. Essential oils from *Cudrania tricuspidata* fruit mutilated the surface morphology and disrupted the cell membrane of *B. cereus* and *E. coli* at 200- 500 µg/mL concentration[4].

Chen et al[9] revealed that the *Litsea cubeba* essential oil treatment against *Cutibacterium acnes* results in wrinkled cells, cell wall distortion and cell rupture. Kumar and Singh[15] revealed that the *Oenanthe javanica* essential oil from aerial part, disrupts the morphology, intracellular structures, increases cell membrane permeability and decreases the membrane potential of *Salmonella* Typhimurium. Essential oils predominantly cause membrane disruption, inhibition of bacterial efflux pump system, generate oxidative stress and leads to intracellular leakage[30].

In our investigation, OJEO showed antiproliferative activity against HaCat cell lines and had IC_{50} value of 0.082 mg/mL. At maximum 5 mg/mL concentration, only 6% viable cells remained. *Zanthoxylum bungeanumi* essential oils evaluated against HaCaT cell exhibited anti-proliferative potential and anti-psoriasis mechanisms[16]. Jugreet et al[13] investigated that *Curcuma longa, Syzygium coriaceum, Cinnamomum camphora, Petroselinum crispum, Pittosporum senacia, Citrus aurantium, Plectranthus amboinicus* and *Syzygium samarangense* essential oils showed cytotoxic activity against HaCat cells and had IC_{50} values of 33.73–250.90 µg/mL. Essential oils exert apoptosis, mitochondrial stress induction and modulation in apoptosis pathways[21]. More than 50% of drugs of cancer originated from medicinal plant i.e. taxol, vinblastine, vincristine etc. are currently used in cancer treatments[13].

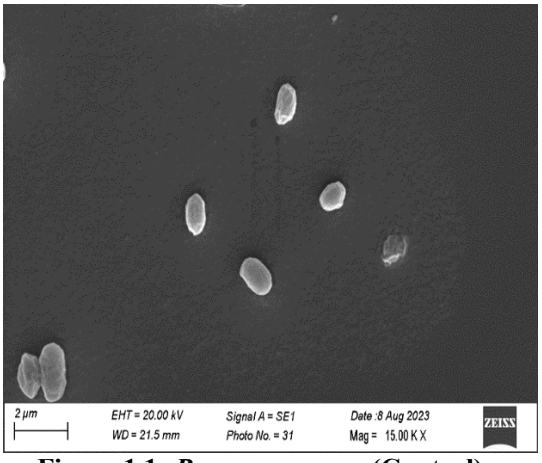


Figure 1.1: *B. cereus* spores (Control)

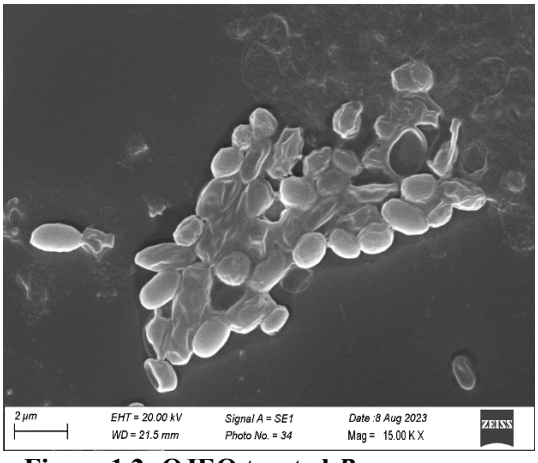


Figure 1.2: OJEO treated *B. cereus* spores

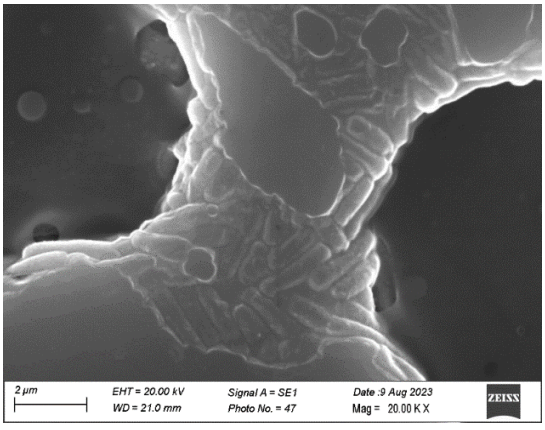


Figure 2.1: *E.coli* (Control)

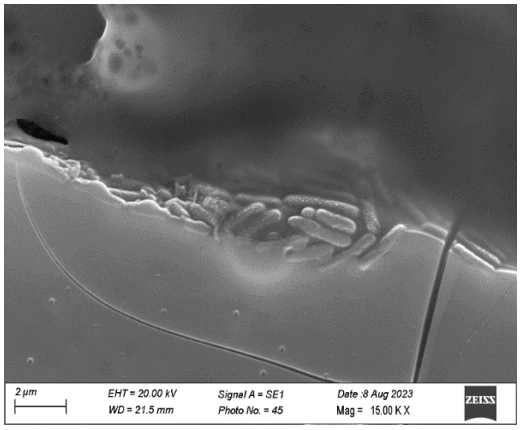


Figure 2.2: OJEO exposed *E.coli* cells

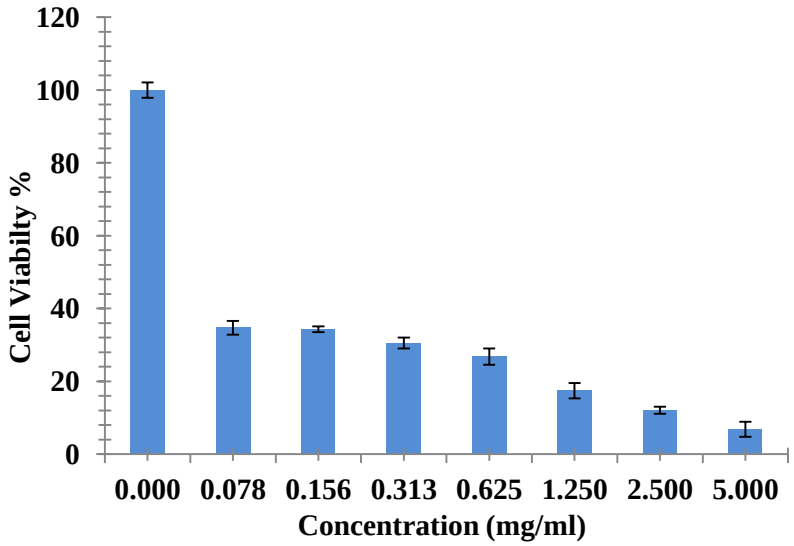


Figure 3: NRU Assay -cell cytotoxicity of OJEO against HaCaT cell line.

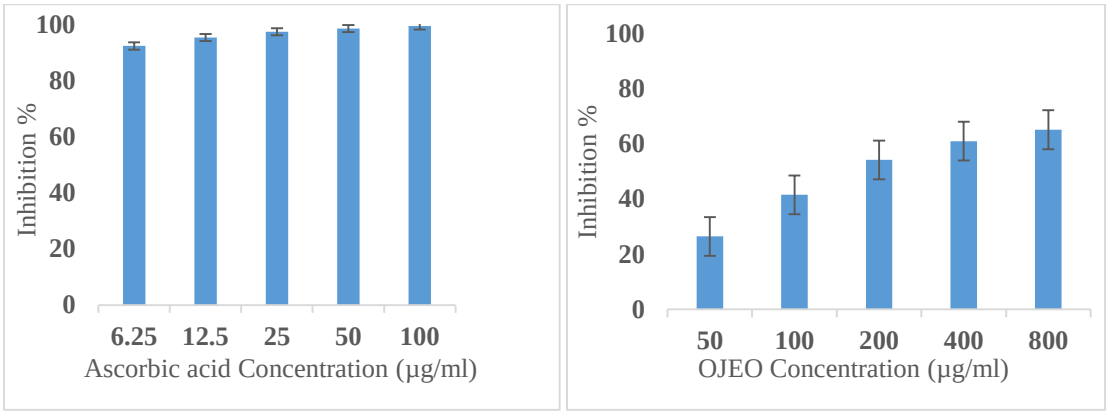


Figure 4: DPPH analysis of Standard (Ascorbic acid) and OJEO at different concentrations.

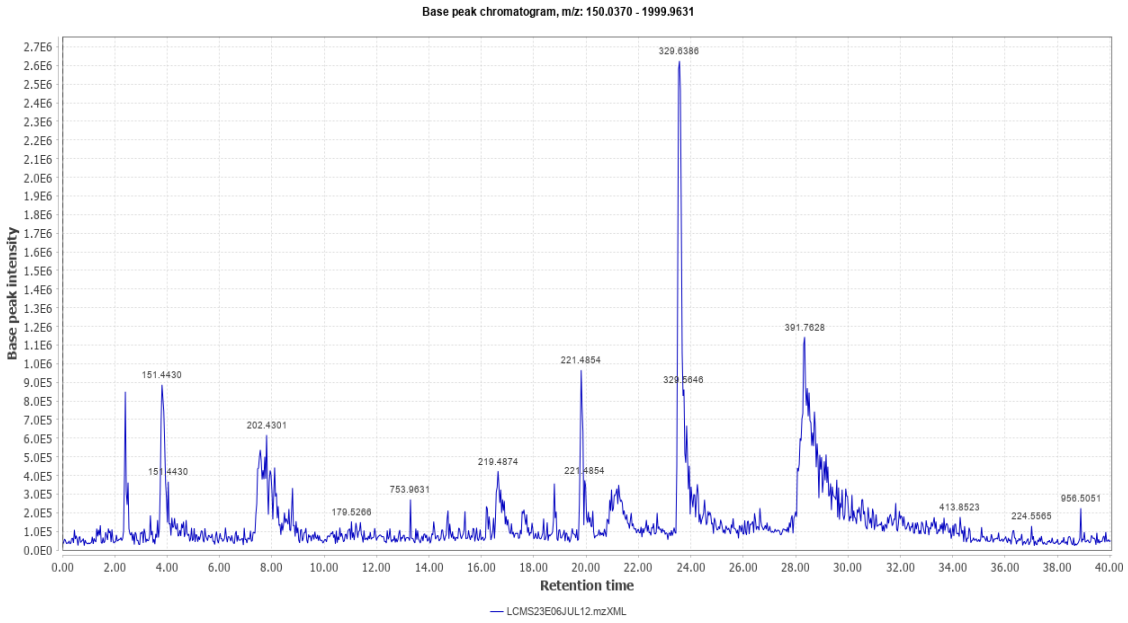


Figure 5: Total Ion Chromatogram (Positive Mode).

Reactive oxygen species (ROS) acts as pivotal components of the immune system when present in low concentrations but the increased ROS rate leads to the destruction of lipids, proteins and DNA molecules and affects the cellular function. ROS generates the oxidative stress which causes cardiovascular disease, Alzheimer′s, Parkinson′s, diabetes,

tumor progression and cancer[31]. OJEO showed radical scavenging activity in DPPH analysis. OJEO inhibits maximum 65.09% radicals at 800 µg/mL concentration. *Mentha piperita, Mentha spicata* and *Mentha gracilis* essential oils are reported to have antioxidant activities in DPPH analysis and their EC_{50} values were 70.29 ± 4.59, 86.51 ± 5.45 and 109.8 ± 6.70[29].

Thymus vulgaris demonstrated DPPH scavenging activity of 32% at 1 mg/mL concentration exposed for 5 min reported by Aebisher et al[2]. Kamal et al[14] reported that *Ammi visnaga* L. essential oils exhibited high antioxidant activity at 600 and 1200 mg/kg in *in vivo* experiment on Swiss albino mice and confirmed the reduction of lipid peroxidation. Essential oils are already investigated to have one of the most common biological activities i.e. antioxidant potential[18]. LC–MS analysis of the OJEO revealed the presence of various chemical components. Tables 2 and 3 present the compound list while figure 5 and 6 shows its total ion chromatogram in positive mode and negative mode.

Smelcerovic et al[23] analyzed the oils of *Achillea millefolium* and *Achillea crithmifolia* using LC-MS technique and revealed that it can screen and identify the components precisely, accurately and rapidly. *Cinnamomum zeylanicum* essential oil's phenolic components were evaluated by LC-HRMS analysis and confirmed the presence of chlorogenic acid, fumaric acid, luteolin-7-rutinoside, luteolin, quillaic acid and many other phenolic compounds[17]. The bioactivity of essential oils depends on chemical constituents and their antagonistic and synergistic effects. Presence of diverse components and lipophilic nature of oils help to easily penetrate intracellularly to exhibit pharmacological and therapeutic activity[10].

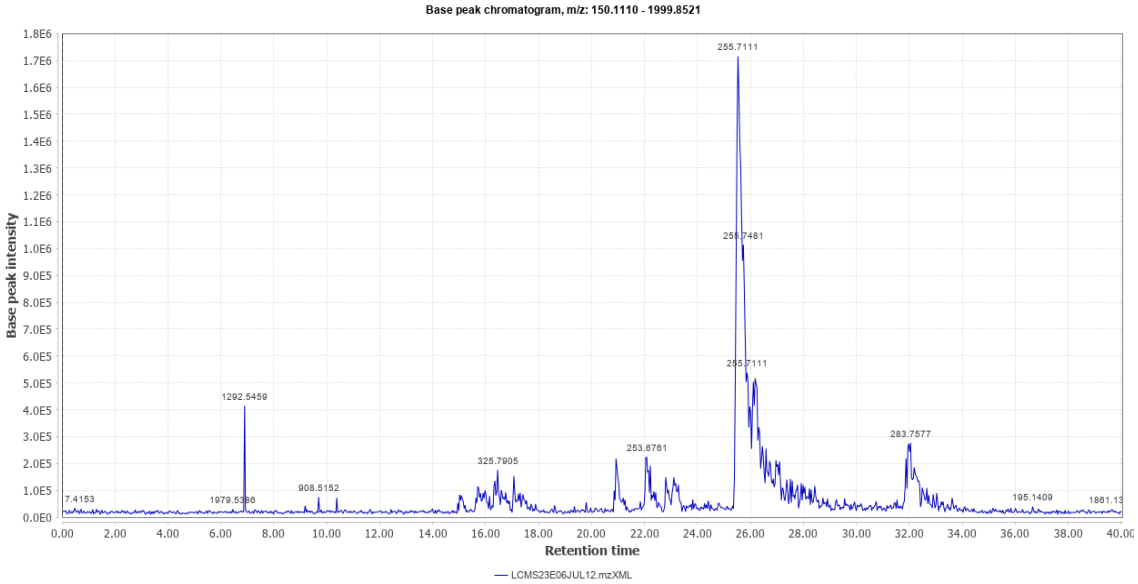


Figure 6: Total Ion Chromatogram (Negative Mode).

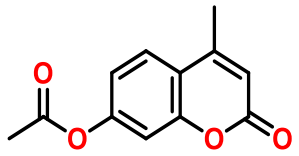
Table 1
Antibacterial potential of OJEO

Bacteria	OJEO (IZD)	Cefotaxime (30 µg) (IZD)	OJEO MIC (µg/mL)	OJEO MBC (µg/mL)
Bacillus cereus	13±0.57	40±1.15	800	1200
Escherichia coli	12±0.57	33.33±1.15	2000	2500

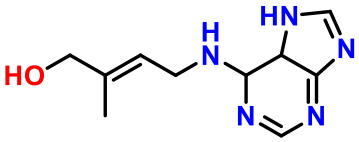
Data are represented as mean± standard error, Inhibition Zone Diameter (IZD) in mm.

Table 2
List of compounds in Positive mode (OJEO)

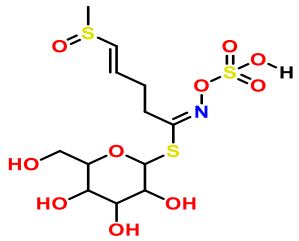
R. Time	Score	Compound Name	Ion	Formula	Exact Mass	Observed Mass	Mass Difference
16.64	0.853	4-Methylumbelliferyl acetate	Positive	$C_{12}H_{10}O_4$	218.057	219.4874	-1.4304
18.79	0.646	4-(Methylsulfinyl)but-3-enylglucosinolate	[M+H]+	$C_{12}H_{21}NO_{10}S_3$	435.49	236.5817	198.9083
19.81	0.936	DL-Dihydrozeatin	Positive	$C_{10}H_{15}N_5O$	221.127	221.4854	-0.3584
23.84	0.665	Scoulerin	Positive	$C_{19}H_{21}NO_4$	327.147	329.7496	-2.6026
29.57	0.454	1-O-b-D-glucopyranosyl sinapate	Positive	$C_{17}H_{22}O_{10}$	386.121	391.7258	-5.6048



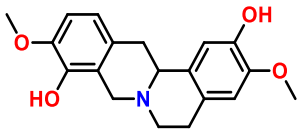
4-Methylumbelliferyl acetate



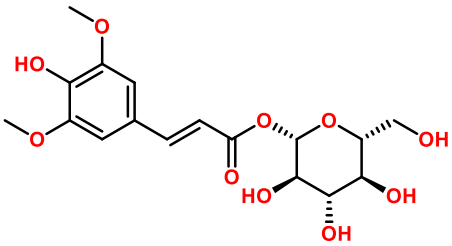
DL-Dihydrozeatin



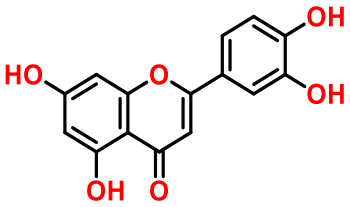
4-(Methylsulfinyl)but-3-enylglucosinolate



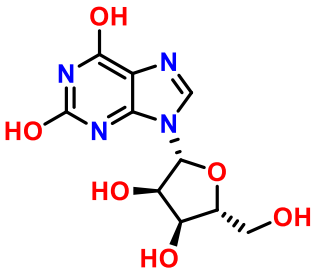
Scoulerin



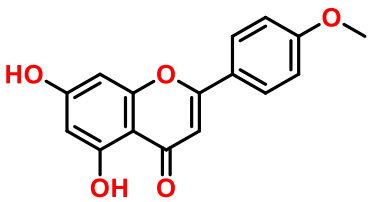
1-O-b-D-glucopyranosyl sinapate



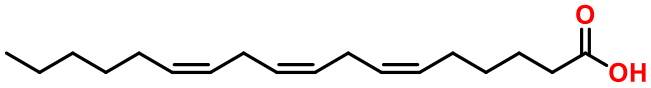
Luteolin



Xanthosine



Acacetin



γ-Linolenic acid

Figure 7: Chemical structures of chemical components in OJEO (LC-MS analysis).

Table 3
List of compounds in Negative mode (OJEO)

R. Time	Score	Compound Name	Ion	Formula	Exact Mass	Observed Mass	Mass Difference
22.80	0.864	γ-Linolenic acid	[M-H]-	$C_{18}H_{30}O_2$	278.43	279.6876	-1.2576
26.18	0.849	Luteolin	Negative	$C_{15}H_{10}O_6$	286.047	281.7596	4.2874
26.24	0.839	Luteolin	Negative	$C_{15}H_{10}O_6$	286.047	281.7596	4.2874
31.98	0.908	Xanthosine	Negative	$C_{10}H_{12}N_4O_6$	284.075	283.7577	0.3173
32.18	0.836	Acacetin	Negative	$C_{16}H_{12}O_5$	284.068	283.7207	0.3473

The present study infers that *Oenanthe javanica* essential oil can be used as antibacterial, antioxidant in preservatives, therapeutics and in pharmaceutical industries. Furthermore, more work is needed to bioprospect *Oenanthe javanica* to explore the natural source of biomolecules for therapeutic elements.

Conclusion

Oenanthe javanica essential oil demonstrated antibacterial activity against the *E.coli* and *B. cereus*. Oil has shown impact on the bacterial morphological organization. Oil expressed antioxidant potential using DPPH assay clearly indicates that it can prevent redox imbalance and also

showed cytotoxic effect against HaCat cell line. LC-MS analysis revealed the presence of diverse chemical constituents. In the present scenario, antibiotics side effects have been commonly observed. Overuse of antibiotics in human diseases and in animal farming causes the emergence of resistant bacterial strains.

Therefore, there is a need for alternative medications for the cure and prevention of diseases with lower mammalian toxicity. Plant based natural derivatives can be a seedbed for active chemical components for developing therapeutics against food borne bacterial pathogens. Future research is impartial to investigate the remedial properties, safety and mechanism of bioactivity of OJEO.

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References

1. Abebe E., Gugsa G. and Ahmed M., Review on major food-borne zoonotic bacterial pathogens, *J. Trop. Med.*, **2020(1)**, 4674235 **(2020)**

2. Aebisher D., Cichonski J., Szpyrka E., Masjonis S. and Chrzanowski G., Essential oils of seven Lamiaceae plants and their antioxidant capacity, *Molecules*, **26**, 3793 **(2021)**

3. Badalamenti N., Maresca V., Di Napoli M., Bruno M., Basile A. and Zanfardino A., Chemical composition and biological activities of Prangos ferulacea essential oils, *Molecules*, **27**, 7430 **(2022)**

4. Bajpai V.K., Sharma A. and Baek K.H., Antibacterial mode of action of Cudrania tricuspidata fruit essential oil, affecting membrane permeability and surface characteristics of food-borne pathogens, *Food Control*, **32**, 582–590 **(2013)**

5. Bauer A.W., Kirby W.M.M., Sherris J.C. and Turck M., Antibiotic susceptibility testing by a standardized single disk method, *Am. J. Clin. Pathol*., **45**, 493–496 **(1966)**

6. Bintsis T., Foodborne pathogens, *AIMS Microbiol*., **3(3)**, 529 **(2017)**

7. Brand-Williams W., Cuvelier M.E. and Berset C.L.W.T., Use of a free radical method to evaluate antioxidant activity, *LWT-Food Sci. Technol.*, **28**, 25–30 **(1995)**

8. Carson C.F., Mee B.J. and Riley T.V., Mechanism of action of Melaleuca alternifolia (tea tree) oil on Staphylococcus aureus determined by time-kill, lysis, leakage and salt tolerance assays and electron microscopy, *Antimicrob. Agents Chemother*., **46**, 1914–1920 **(2002)**

9. Chen J., Zhang J., Zhu L., Qian C., Tian H., Zhao Z., Jin L. and Yang D., Antibacterial activity of the essential oil from Litsea cubeba against Cutibacterium acnes and the investigations of its potential mechanism by gas chromatography-mass spectrometry metabolomics, *Front. Microbiol*., **13**, 823845 **(2022)**

10. de Sousa D.P., Damasceno R.O.S., Amorati R., Elshabrawy H.A., de Castro R.D., Bezerra D.P., Nunes V.R.V., Gomes R.C. and Lima T.C., Essential oils: Chemistry and pharmacological activities, *Biomolecules*, **13**, 1144 **(2023)**

11. Han Y.Q., Huang Z.M., Yang X.B., Liu H.Z. and Wu G.X., In vivo and in vitro anti-hepatitis B virus activity of total phenolics from Oenanthe javanica, *J. Ethnopharmacol*., **118**, 148–153 **(2008)**

12. Heinrich M., Ethnobotany and its role in drug development, *Phytother. Res*., **14**, 479–488 **(2000)**

13. Jugreet B.S., Lall N., Lambrechts I.A., Reid A.M., Maphutha J., Nel M., Hassan A.H., Khalid A., Abdalla A.N., Van B.L. and Mahomoodally M.F., *In vitro* and *in silico* pharmacological and cosmeceutical potential of ten essential oils from aromatic medicinal plants from the Mascarene Islands, *Molecules*, **27**, 8705 **(2022)**

14. Kamal F.Z., Stanciu G.D., Lefter R., Cotea V.V., Niculaua M., Ababei D.C., Ciobica A. and Ech-Chahad A., Chemical composition and antioxidant activity of Ammi visnaga L. essential oil, *Antioxidants*, **11**, 347 **(2022)**

15. Kumar R. and Singh P., Assessment of antibacterial, antioxidant, cytotoxic properties and chemical composition of Oenanthe javanica (Blume) DC. essential oil, *J. Herb. Med*., **49**, 100982 **(2025)**

16. Li K., Zhou R., Jia W.W., Li Z., Li J., Zhang P. and Xiao T., Zanthoxylum bungeanum essential oil induces apoptosis of HaCaT human keratinocytes, *J. Ethnopharmacol*., **186**, 351–361 **(2016)**

17. Mutlu M., Bingol Z., Uc E.M., Köksal E., Goren A.C., Alwasel S.H. and Gulcin İ., Comprehensive metabolite profiling of cinnamon (Cinnamomum zeylanicum) leaf oil using LC-HR/MS, GC/MS and GC-FID: Determination of antiglaucoma, antioxidant, anticholinergic and antidiabetic profiles, *Life*, **13**, 136 **(2023)**

18. Mutlu-Ingok A.L., Devecioglu D., Dikmetas D.N., Karbancioglu-Guler F. and Capanoglu E., Antibacterial, antifungal, antimycotoxigenic and antioxidant activities of essential oils: An updated review, *Molecules*, **25**, 4711 **(2020)**

19. Önder S., Periz Ç.D., Ulusoy S., Erbaş S., Önder D. and Tonguç M., Chemical composition and biological activities of essential oils of seven cultivated Apiaceae species, *Sci. Rep*., **14**, 10052 **(2024)**

20. Rodrigues R.M., Stinckens M., Ates G. and Vanhaecke T., Neutral Red Uptake Assay to Assess Cytotoxicity *In Vitro*, In Cell Viability Assays: Methods and Protocols, Springer US, New York, 237–245 **(2023)**

21. Saad N.Y., Muller C.D. and Lobstein A., Major bioactivities and mechanism of action of essential oils and their components, *Flavour Fragr. J*., **28**, 269–279 **(2013)**

22. Siddiqui A.J. et al, Plants in anticancer drug discovery: from molecular mechanism to chemoprevention, *Biomed. Res. Int*., **2022(1)**, 5425485 **(2022)**

23. Smelcerovic A., Lamshoeft M., Radulovic N., Ilic D. and Palic R., LC–MS Analysis of the essential oils of Achillea millefolium and Achillea crithmifolia, *Chromatographia*, **71**, 113–116 **(2010)**

24. Süntar I., Importance of ethnopharmacological studies in drug discovery: role of medicinal plants, *Phytochem. Rev*., **19**, 1199–1209 **(2020)**

25. Tamokou J.D.D., Mbaveng A.T. and Kuete V., Antimicrobial activities of African medicinal spices and vegetables, In Medicinal Spices and Vegetables from Africa, Academic Press, 207–237 **(2017)**

26. Ušjak L., Stojković D., Carević T., Milutinović V., Soković M., Niketić M. and Petrović S., Chemical analysis and investigation of antimicrobial and antibiofilm activities of Prangos trifida (Apiaceae), *Antibiotics*, **13**, 41 **(2024)**

27. Wadud A., Prasad P.V.V., Rao M.M. and Narayana A., Evolution of Drug: A Historical Perspective, *Bulletin of the Indian Institute of History of Medicine (Hyderabad)*, **37(1)**, 69–80 **(2007)**

28. Wayne P.A., Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, Approved Standard M7-A7, Clinical and Laboratory Standards Institute **(2006)**

29. Wu Z., Tan B., Liu Y., Dunn J., Martorell Guerola P., Tortajada M., Cao Z. and Ji P., Chemical composition and antioxidant properties of essential oils from peppermint, native spearmint and scotch spearmint, *Molecules*, **24**, 2825 **(2019)**

30. Yang S.K., Tan N.P., Chong C.W., Abushelaibi A., Lim S.H.E. and Lai K.S., The missing piece: Recent approaches investigating the antimicrobial mode of action of essential oils, *Evol. Bioinform*., **17**, 117693432093839 **(2021)**

31. Zahra K.F., Lefter R., Ali A., Abdellah E.C., Trus C., Ciobica A. and Timofte D., The involvement of the oxidative stress status in cancer pathology: A double view on the role of the antioxidants, Oxid. Med. *Cell Longev*., https://doi.org/10.1155/2021/9965916 **(2021)**

32. Zhang L., Song J., Kong L., Yuan T., Li W., Zhang W., Hou B., Lu Y. and Du G., The strategies and techniques of drug discovery from natural products, *Pharmacol. Ther*., **216**, 107686 **(2020)**.

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